THE TOXICITY OF GALACTOSE AND MANNOSE FOR GREEN PLANTS AND THE ANTAGONISTIC ACTION OF OTHER SUGARS TOWARD THESE[1]

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In experiments concerned primarily with the utilization of certain sugars by certain green plants (Knudson, 1915, 1916), the noteworthy fact developed that, while other sugars may be of benefit, galactose is toxic. The injurious effect was manifested in a killing of the root or a retardation of root growth, depending upon the concentration of galactose employed. It was observed, furthermore, that glucose can antidote the toxicity of galactose, but this antagonism occurs only when the glucose is present at a concentration equal to or greater than that of the galactose. So far as the writer has been able to determine, this is the only recorded case of a hexose sugar being injurious to plants and of antagonism among the sugars.

In view of the fact that glucose exhibited such a marked antagonistic action toward galactose, it seemed advisable to extend further the investigation to include various other sugars. A considerable number of experiments have been made to this end and the paper here presented records briefly the results obtained.

Methods.—For the experiments either Canada field pea (*Pisum arvense* L.) or wheat (*Triticum sativum* L.) was used. The plants were grown in all cases under conditions insuring freedom from microorganisms. For this purpose the plants were grown in culture tubes 200 mm. × 20 mm. in size, on a nutrient agar medium. Pfeffer's nutrient solution, slightly modified, was made up as follows: $Ca(NO_3)_2$, 4 grams; KNO_3, 1 gram; K_2HPO_4, 1 gram; KCl, 0.5 gram; $MgSO_4$, 0.5 gram; $FeCl_3$, 20 milligrams; distilled water, 12 liters. The agar used had been previously rinsed three times in distilled water and then air-dried. One percent of agar was used. The medium is faintly alkaline to methyl red. The different sugars were dissolved in this medium and stock solutions were made up of double the concentration of sugar used in the experiments. Dilution of the sugar was effected

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by addition to the nutrient solution or by addition of another sugar solution. For example, to obtain a solution containing 0.25 mol. galactose + 0.025 mol. saccharose, it was necessary to mix equal parts of 0.5 mol. galactose and 0.5 saccharose. The volume of the medium in each tube was 25 cc. Sterilization was effected by autoclaving at fifteen pounds pressure for fifteen minutes.

All the sugars used, with the exception of arabinose, were supplied by Dr. C. S. Hudson, in charge of the carbohydrate laboratory, U. S. Bureau of Chemistry, and are stated by him to be of very high purity. The arabinose used was a Merck reagent.

Character of the Injury.—The injurious action of galactose is made evident first in the roots. The primary root coming in contact with the agar may first become brown and in a few days death results. In other cases the tip of the root is killed and this stimulates the production of a large number of lateral roots, the tips of which, on coming in contact with the agar medium, are soon killed. A short primary root with many laterals results, the appearance of which is somewhat centipedal. Two plants in the same culture may, however, vary in the manner of injury, and the presence of certain sugars may alter the extent of the injury.

For the sake of clearness and definiteness, it seems desirable to describe the injury by a numerical system as well as by root lengths. Accordingly the following key is given: 0, no injury; 1, primary root tip killed, laterals not injured; 2, the primary root tip may be killed, but the laterals may attain a length of a few centimeters and then growth is stopped or the roots are killed; 3, the primary root may penetrate the agar, but becomes brownish and five or six centimeters long; 4, the primary root may attain a length of a few centimeters, but becomes brown in color and the laterals do not grow beyond 0.5 cm.; 5, the primary root tip is killed and all laterals suffer likewise; 6, the primary root is entirely killed.

Antagonistic Action.—In the following experiment the galactose was supplied at a concentration of 0.025 mol. and the other sugars were used at the same concentration. In order to demonstrate conclusively that the total concentration was not responsible for any toxicity, a few cultures were made with the nontoxic sugars supplied at 0.05 mol. The experiment was begun on January 29, 1917, and concluded on February 13, 1917. The cultures were placed in the greenhouse and grown in the light. All cultures were made in trip-

licate, but contamination or failure to germinate caused a loss of some of the cultures. The seed were sterilized by immersion in a solution of calcium hypochlorite (calcium oxychloride, Baker) according to the method of Wilson (1915). The peas were treated for two hours and the wheat for five hours. The results are given in Table 1.

TABLE 1

Influence of Sugars on the Toxicity of Galactose

The Concentration of Each Sugar Equals 0.025 Mol.	Length of Primary Root (Cm.)	Average Length of Lateral Root (Cm.)	Length of Top (Cm.)	Class of Injury
Galactose (3 cultures)	1	1/2	6	5
Galactose (2 cultures)	0	0	3	6
Glucose (3 cultures)	10	8	15	0
Levulose (3 cultures)	8	8	14	0
Arabinose (1 culture)	10	7	15	0
Saccharose (3 cultures)	9	8	14	0
Maltose (3 cultures)	10	8	13	0
Raffinose (2 cultures)	9	8	14	0
Pfeffers, no sugar (3 cultures)	10	8	14	0
Galactose+glucose (3 cultures)	9	7	13	0
Galactose+levulose (2 cultures)	5 1/2	1/4	10 1/2	4
Galactose+levulose (1 plant)	7	0	....	5
Galactose+saccharose (3 cultures)	3	7	13	1
Galactose+saccharose (1 culture)	10	9	14	0
Galactose+lactose (3 cultures)	1	1/2	9	5
Galactose+maltose (2 cultures)	1	1/2	10	5
Galactose+raffinose	1	1/2	6	5

From the table it will be noted that the toxicity of galactose is prevented by glucose or saccharose, the former being slightly more effective than the latter since the primary root is not killed in the presence of glucose. None of the other sugars are effective in preventing the injurious action of galactose, although in the presence of levulose the primary root may continue its growth to a limited extent. Representative cultures are shown in Fig. 1.

All of the preceding experiments except those with levulose were repeated and similar results were obtained.

In some earlier experiments (Knudson, 1916) it was noted that glucose does not antidote galactose if the concentration of the former is less than that of the latter. It was thought that some relation might be found between concentrations and antagonistic action. Accordingly the galactose was supplied in each case at a concentration of 0.0125 mol. solution, and the other sugars used at double this concen-

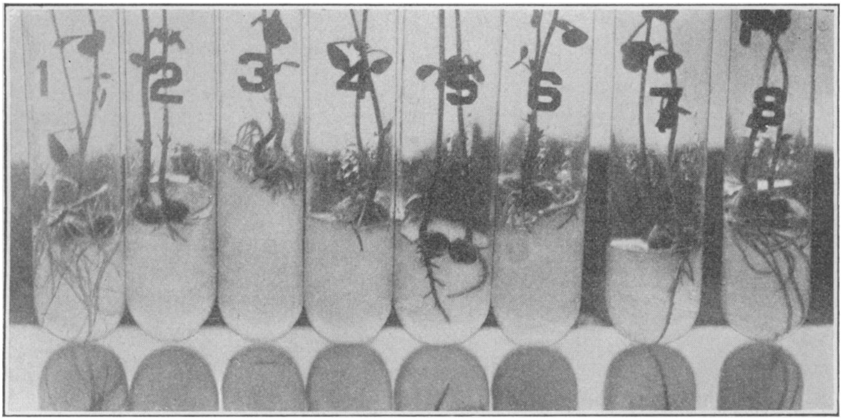

FIG. 1. 1. Galactose .025 mol. + saccharose .025 mol.
2. " " " + maltose .025 "
3. " " " + raffinose .025 "
4. " " " + lactose .025 "
5. " " " + arabinose .025 "
6. " " "
7. " " " + levulose .025 "
8. Pfeffer's solution. No sugar.

TABLE 2

Influence of Sugars on the Toxicity of Galactose

Culture Solution	Length of Primary Root (Cm.)	Average Length of Lateral Root (Cm.)	Length of Top (Cm.)	Class of Injury
Galactose .0125 mol. (2 cultures)	1	0.5	7	5
Galactose .0125 mol.+glucose .025 mol. (1 culture)	9	8	15	0
Galactose .0125 mol.+lactose .025 mol. (1 culture)	7	1.5	12	3
Galactose .0125 mol.+levulose .025 mol. (1 culture)	3	6	13	3
Galactose .0125 mol.+arabinose .025 mol. (1 culture)	5	1	13	4
Galactose .0125 mol.+saccharose .025 mol. (1 culture)	2	8	15	1
Galactose .0125 mol.+lactose .025 mol. (2 cultures)	6	0.5	10	4
Galactose .0125 mol.+lactose .025 mol. (2 cultures)	1	1	12	5
Galactose .012 mol.+maltose .025 mol. (2 cultures)	1	0.5	12	5
Galactose .0125 mol.+raffinose .025 mol. (2 cultures)	1	0.5	10	5

tration, or 0.025 mol. It was noted that 0.0125 mol. galactose is as toxic as 0.025 mol. galactose. The results are given in Table 2. Since there was variation in some of the series, the results of individual cultures are recorded in these cases; for plants that were alike, the averages are recorded.

In general the results are similar to the preceding experiment, though levulose is somewhat more effective than with the higher concentrations of galactose. Cultures are represented in Fig. 2.

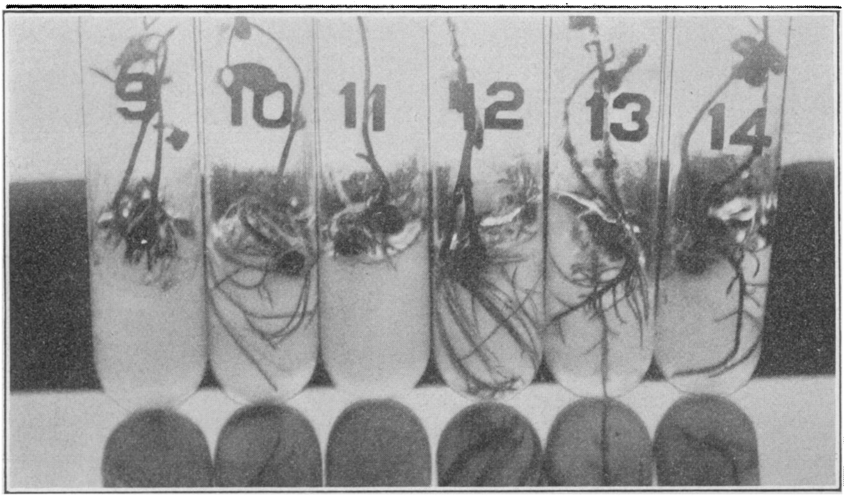

FIG. 2. 9. Galactose .0125
10. " + saccharose .025
11. " + raffinose .025
12. " + glucose .025
13. " + levulose .025
14. " + lactose .025
15. Lactose .05
16. Pfeffer's solution. No sugar.

Toxicity of Mannose.—In the course of certain experiments on the use of various sugars by vetch (*Vicia villosa*) grown in water cultures, it was noted that mannose, supplied at a concentration of 0.025 mol., killed the tips of roots that came into contact with the solution. Experiments were then made with pea and wheat to determine whether the effect was consistent, and agar cultures were used as in the ex-

periments previously described with galactose. It was found that mannose at a concentration of 0.025 mol. behaved very much as did galactose, with the possible exception that the browning of the roots was not so intense as with galactose. The same general effect, however, was noted as is evident in Figs. 3 and 4.

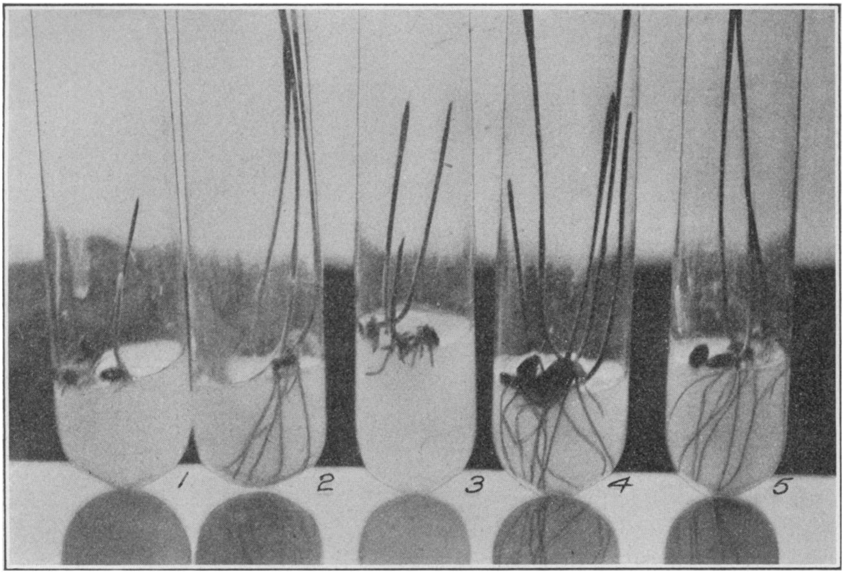

FIG. 3. 1. Galactose .025 mol.
2. Mannose .025 "
3. Mannose .025 " + galactose .025 mol.
4. Mannose .025 " + glucose .025 "
5. Pfeffer's solution. No sugar.

Antagonistic Action.—Since glucose and saccharose had been found to antidote effectively galactose, these two sugars were tested with respect to their preventing the toxicity of mannose. Test-tube cultures were used as for the galactose experiments, the amount of the medium being 25 cc. Both peas and wheat were used. The cultures were permitted to grow for two weeks, and the data were then recorded. All cultures were in duplicate, and those for pea, with the exception of the cultures containing saccharose, were repeated.

The cultures employed were as follows:

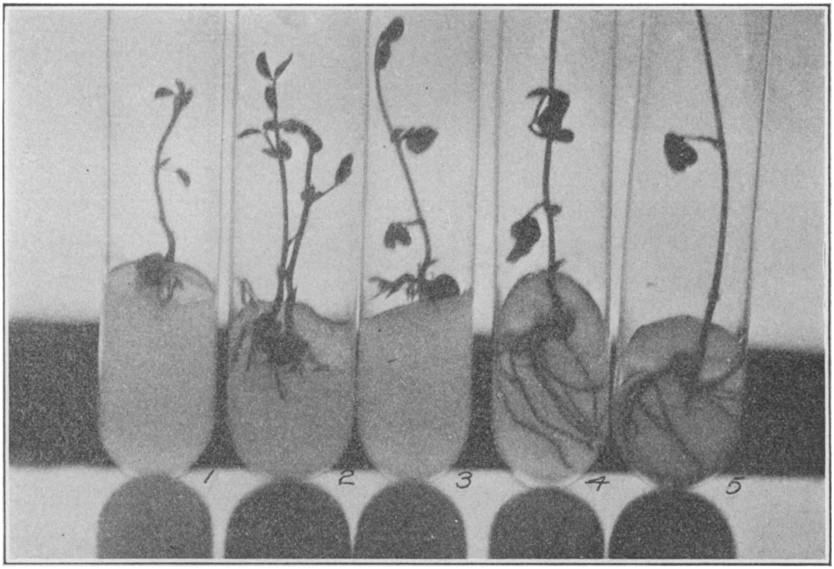

FIG. 4. 1. Galactose .025 mol.
2. " .025 " + mannose .025 mol.
3. Mannose .025 mol.
4. " .025 " + glucose .025 mol.
5. " .025 " + saccharose .025 mol.

1. Galactose .025 mol.
2. Mannose .025 mol.
3. Mannose .025 mol. + galactose .025 mol.
4. Mannose .025 mol. + glucose .025 mol.
5. Mannose .025 mol. + saccharose .025 mol.
6. Glucose .025 mol.
7. Saccharose .025 mol.
8. Pfeffer's solution alone—no sugar.

Both with wheat and with peas it was found that the toxicity of mannose is antidoted by either glucose or saccharose. Mutual antagonism was not found between galactose and mannose. The plants grown in the other solutions were in every way normal. Representative cultures are shown in Figs. 3 and 4.

Discussion.—The hexose sugars glucose, mannose, and galactose are stereoisomers. All of them are used by various fungi, and mannose is as readily fermented by yeasts as is glucose. Galactose,

however, is fermented with greater difficulty, and it is suggested (Armstrong, 1912) that perhaps a different mechanism is involved in its fermentation. Mannose has a common enolic form with glucose and fructose, and any one of the three may be converted into any other under the influence of alkalies. It is therefore all the more surprising to find mannose behaving similarly to galactose and not like glucose.

In a previous paper it was suggested that the toxicity of galactose might be due to its oxidation products. The first oxidation products of glucose and galactose are gluconic and galactonic acids. Various cultures were made with Canada field pea in which the effect of calcium galactate and calcium gluconate was to be noted. The experiments were made as were those previously described. In no case was any injurious action of calcium galactate noted.

It is not yet possible to offer any explanation accounting for the toxicity of the two sugars. An explanation of antagonism is suggested by the phenomenon commonly observed with fungi, namely, the election of organic substances, whereby if two organic substances are offered only one may be absorbed. Various cases of this nature have been reported even for stereoisomeric compounds. According to this view, in a mixture of glucose and galactose, the toxicity of the latter would be prevented because of the absorption of glucose and the nonabsorption of galactose, and a similar condition would hold for a mixture of saccharose and galactose. The failure of the other sugars to antagonize the toxicity of galactose would be due to their inability to prevent the absorption of the galactose. Work is being continued on this subject.

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